

495
|

human eNOS:	AAKGTGITRKKT	-FKEVANAVKISASL	MGTVMASRVKA (SEQ ID NO: 2)
bovine eNOS:	ATKGAGITRKKT	-FKEVANAVKISASL	MGTLMASRVKA (SEQ ID NO: 3)
human iNOS:	DEK-RRPKRREI	PLKVLVKAVLFACML	MRKTMASRVRV (SEQ ID NO: 4)
rat iNOS:	DEK-LRPRRREI	RFTVLVKAVFFASVL	MRKVMASRVRA (SEQ ID NO: 5)
mouse iNOS:	NEK-LRPRRREI	RFRVLVKVFFASML	MRKVMASRVRA (SEQ ID NO: 6)
human nNOS:	GTNGTPTKRRAI	GFKKLAEAVKFSACL	MGQAMASRVKA (SEQ ID NO: 7)
rat nNOS:	GTNGTPTKRRAI	GFKKLAEAVKFSACL	MGQAMASRVKA (SEQ ID NO: 8)

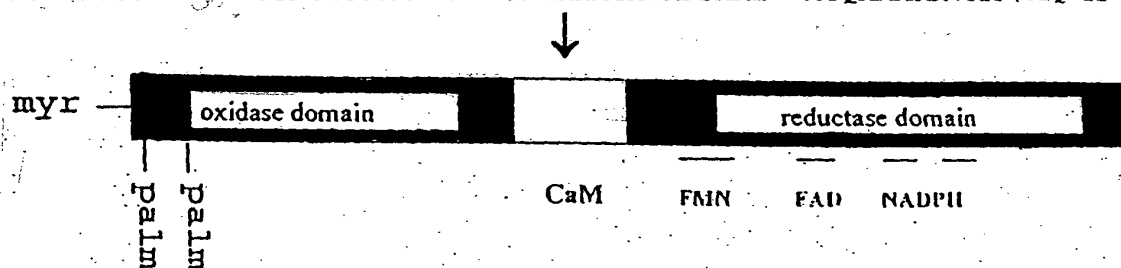


FIGURE 1

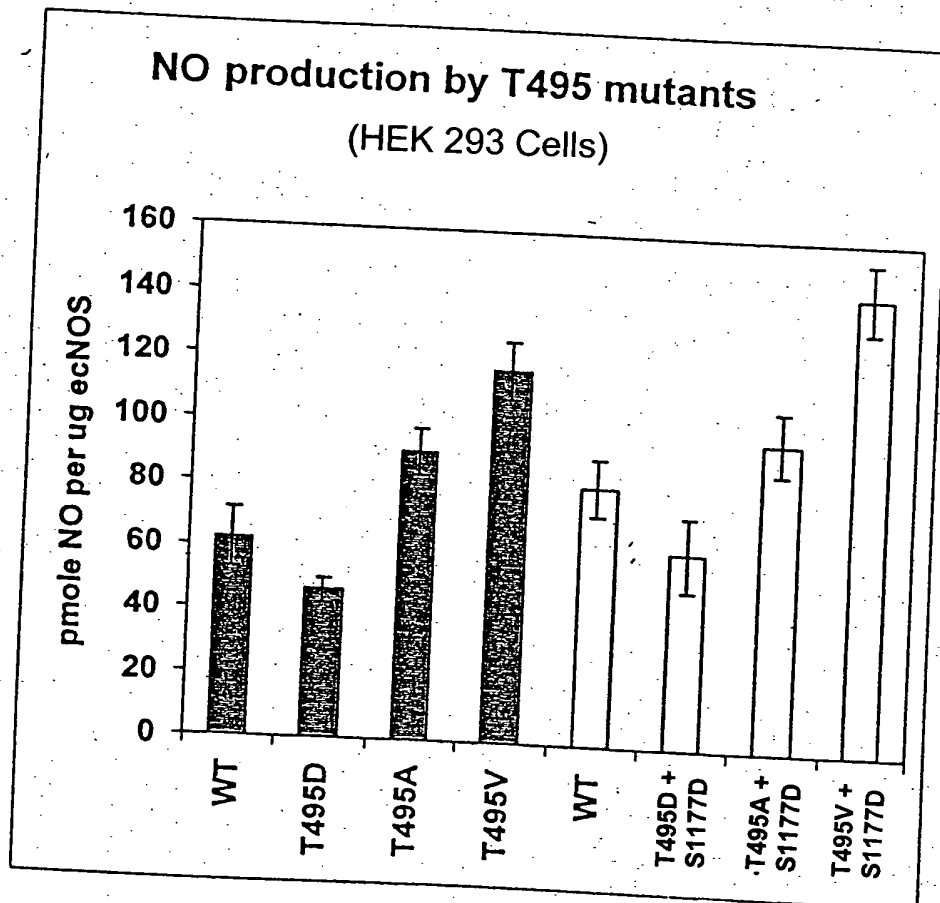
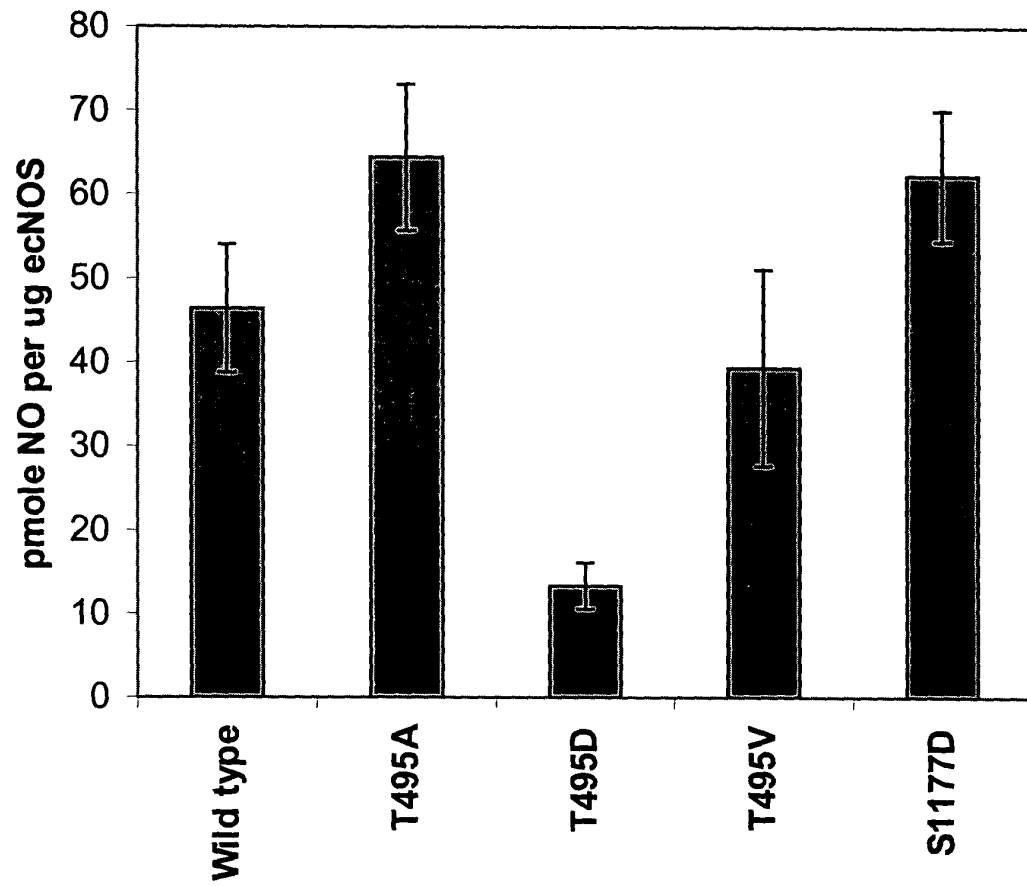


FIGURE 2

3/28

FIGURE 3

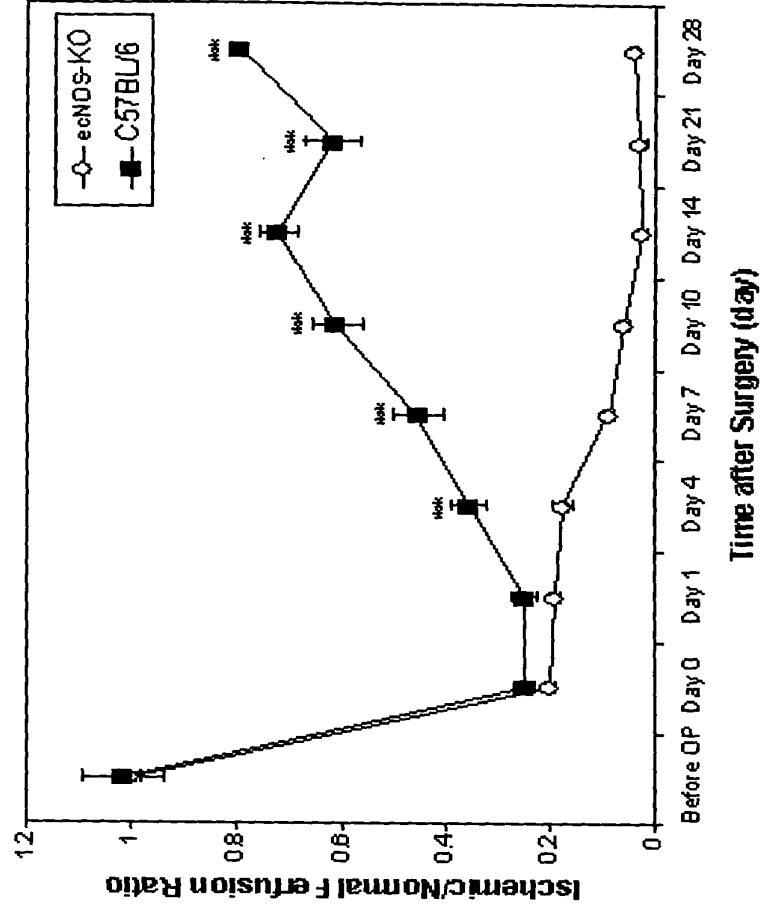
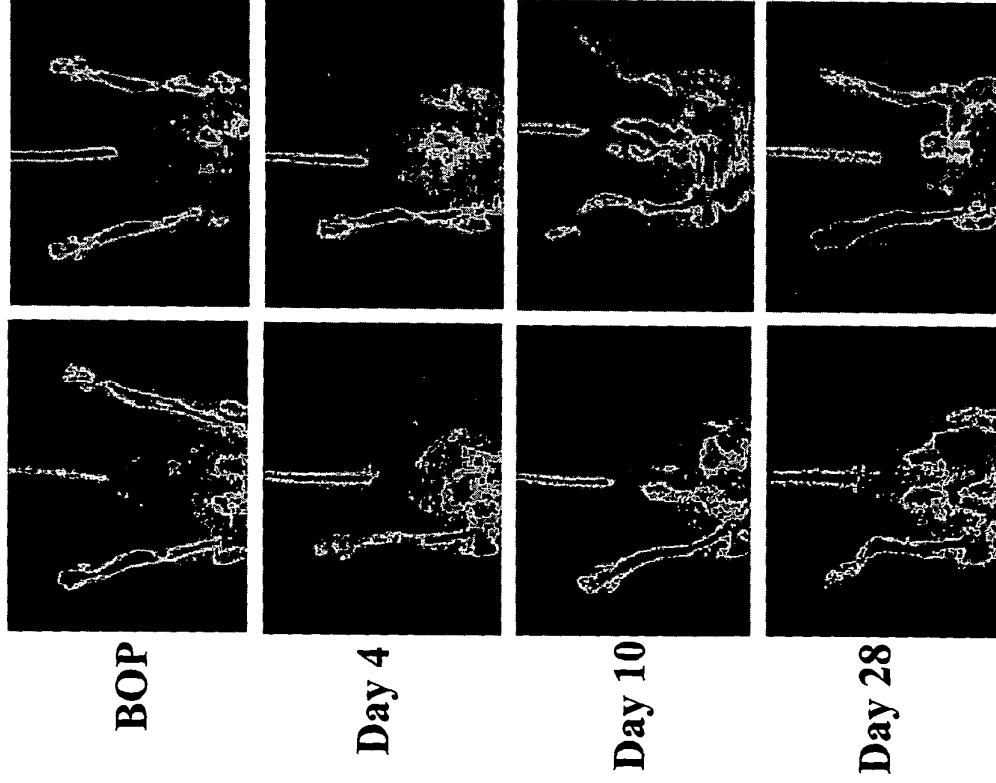


4/28

FIGURE 4: Wt/ecNOS-KO LDPI Flow

C57BL/6

ecNOS-KO



5/28

FIGURE 5: WT/ecNOS-KO gross pathological changes

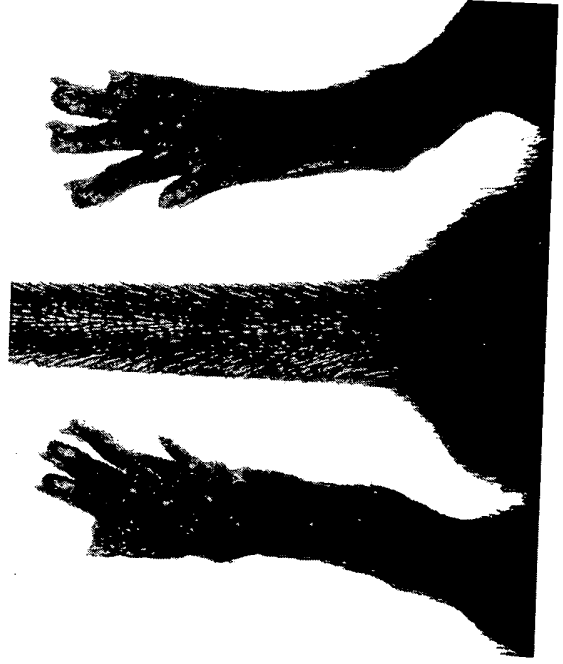
Day 0



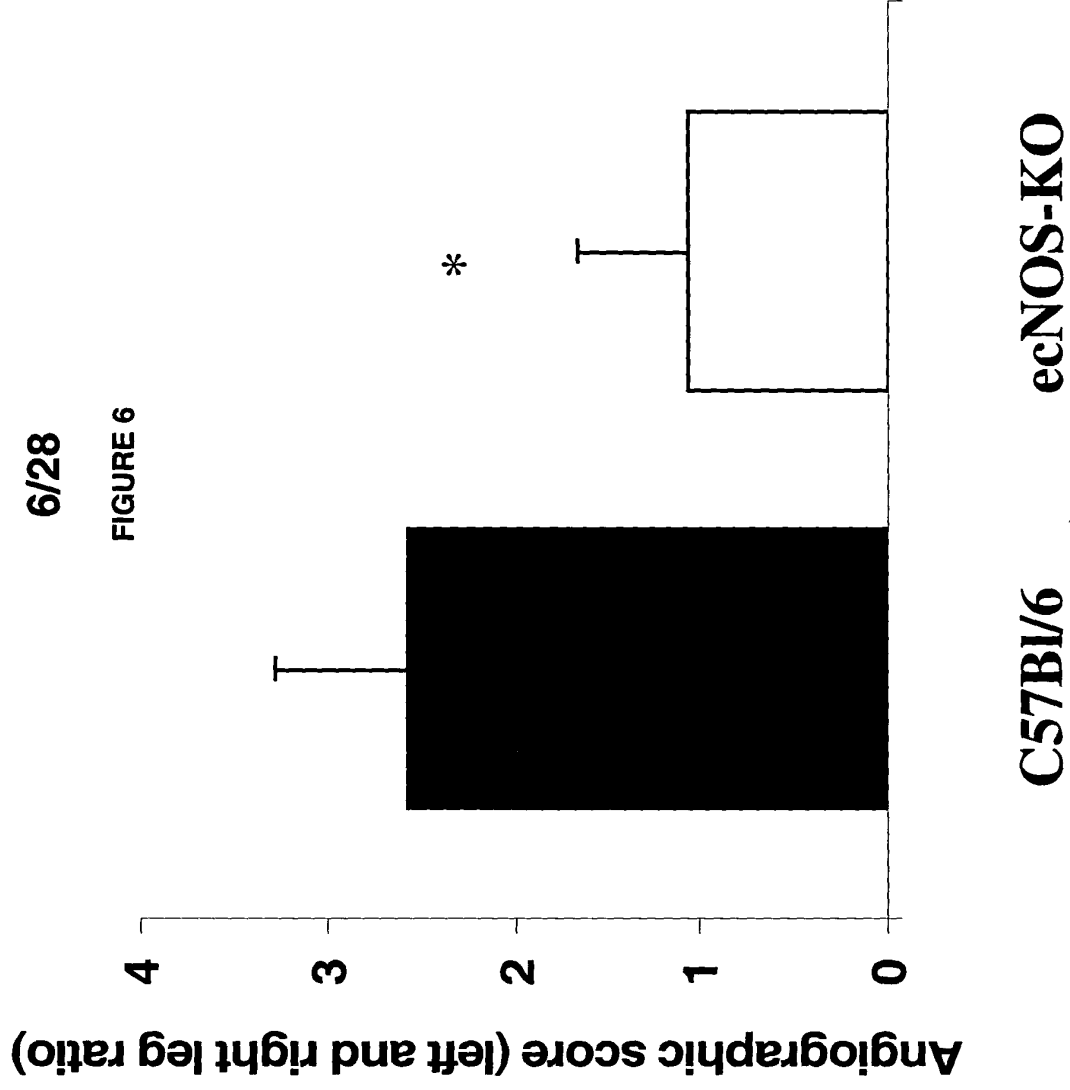
Day 4



ecNOS-KO



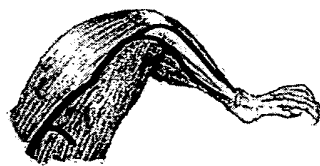
C57BL/6



7/28

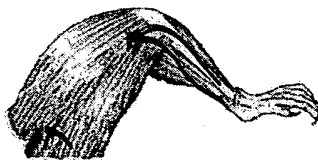
Figure 7: Different Surgical Procedures for CLJ

Normal
Anatomy



Surgical
Procedure

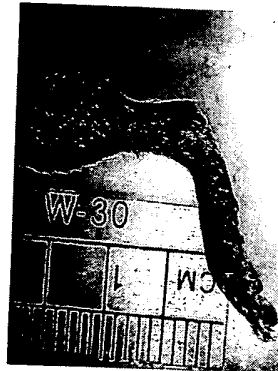
Whole Fem. A. & V.
Resection



Whole Fem. A.
Resection



Segmental Fem. A.
Resection



Gross
Pathology



LDPI Flow

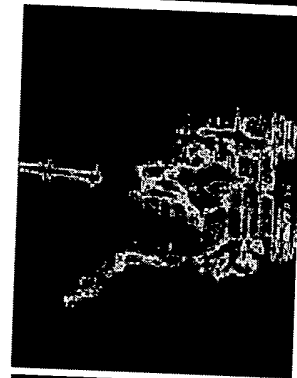
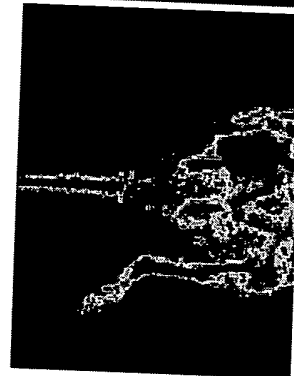
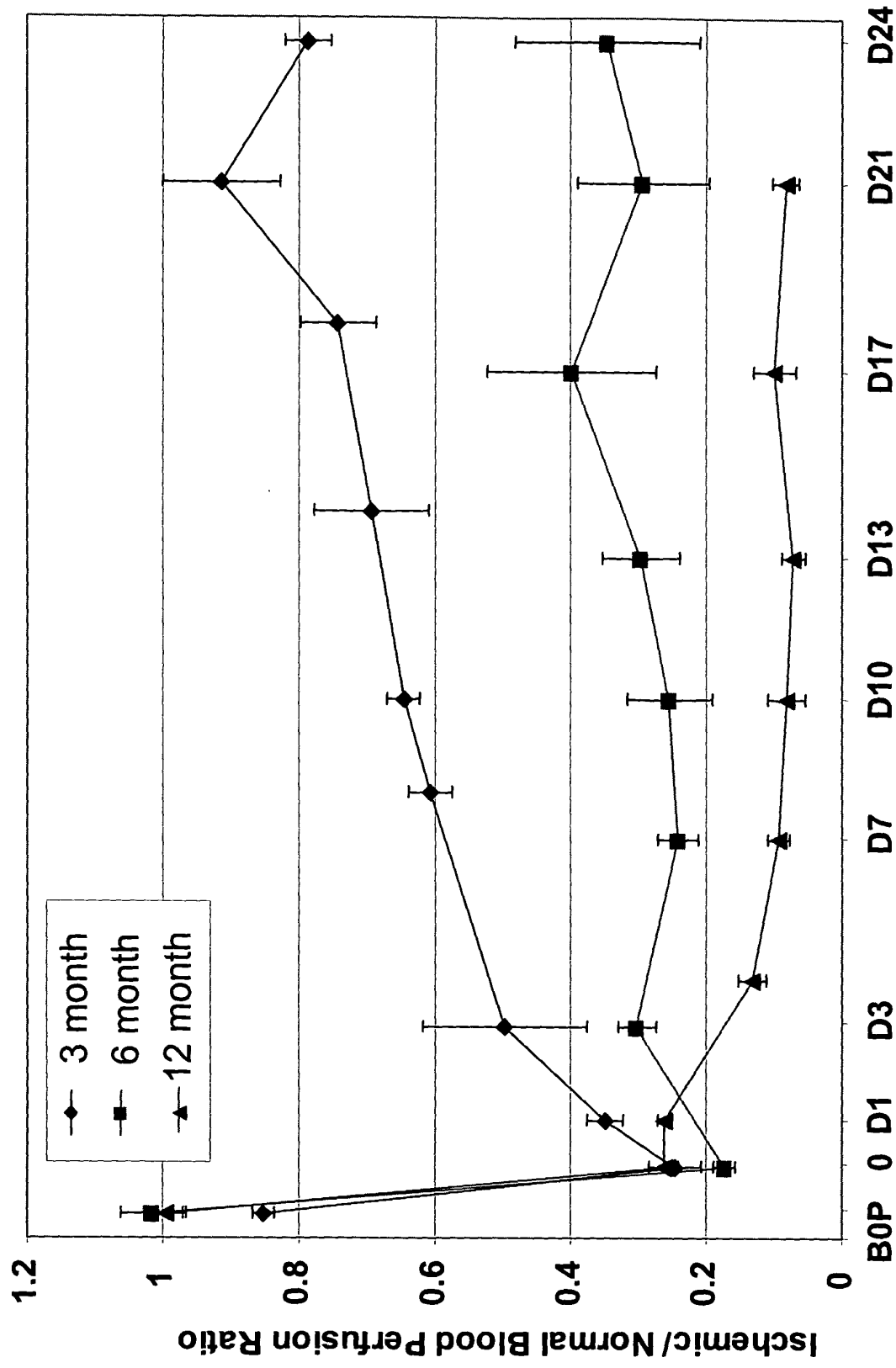
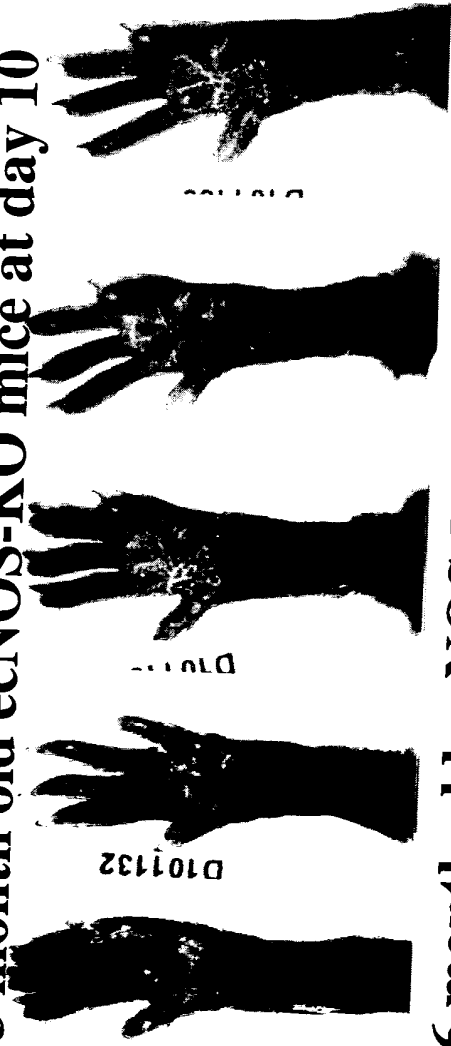


FIGURE 8: Effect of age on spontaneous blood flow recovery



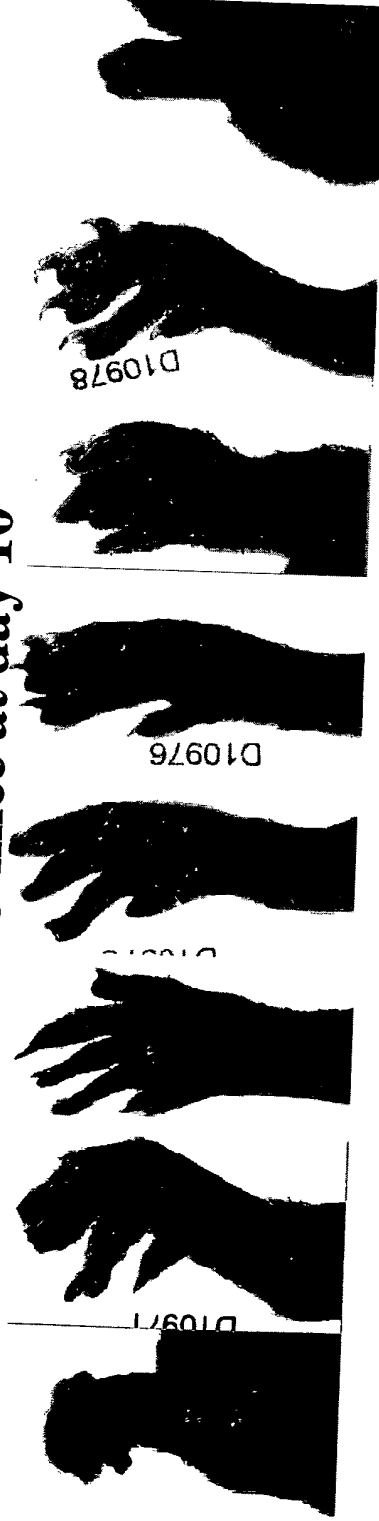
3 month old ecNOS-KO mice at day 10



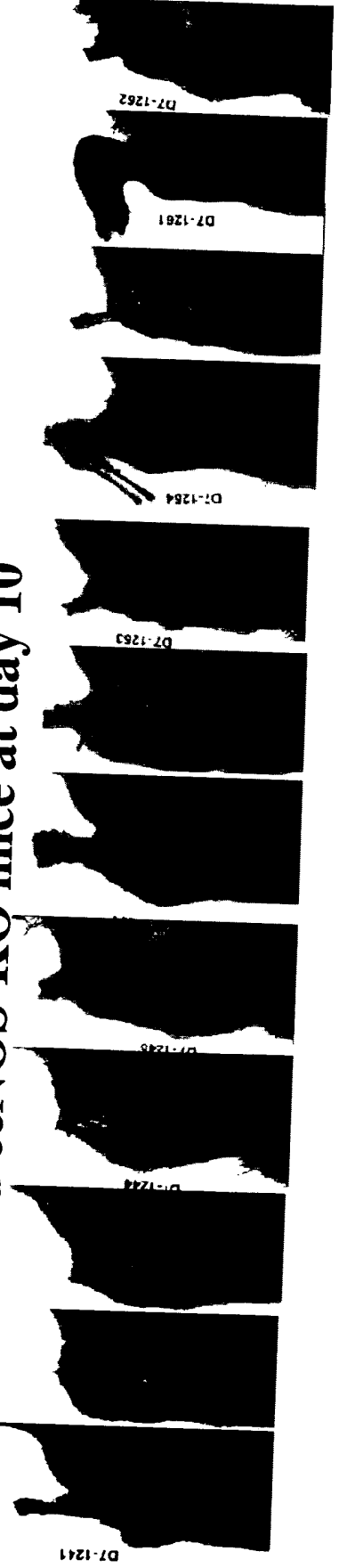
9/28

FIGURE 9: Effect of age on ischemic damage.

6 month old ecNOS-KO mice at day 10

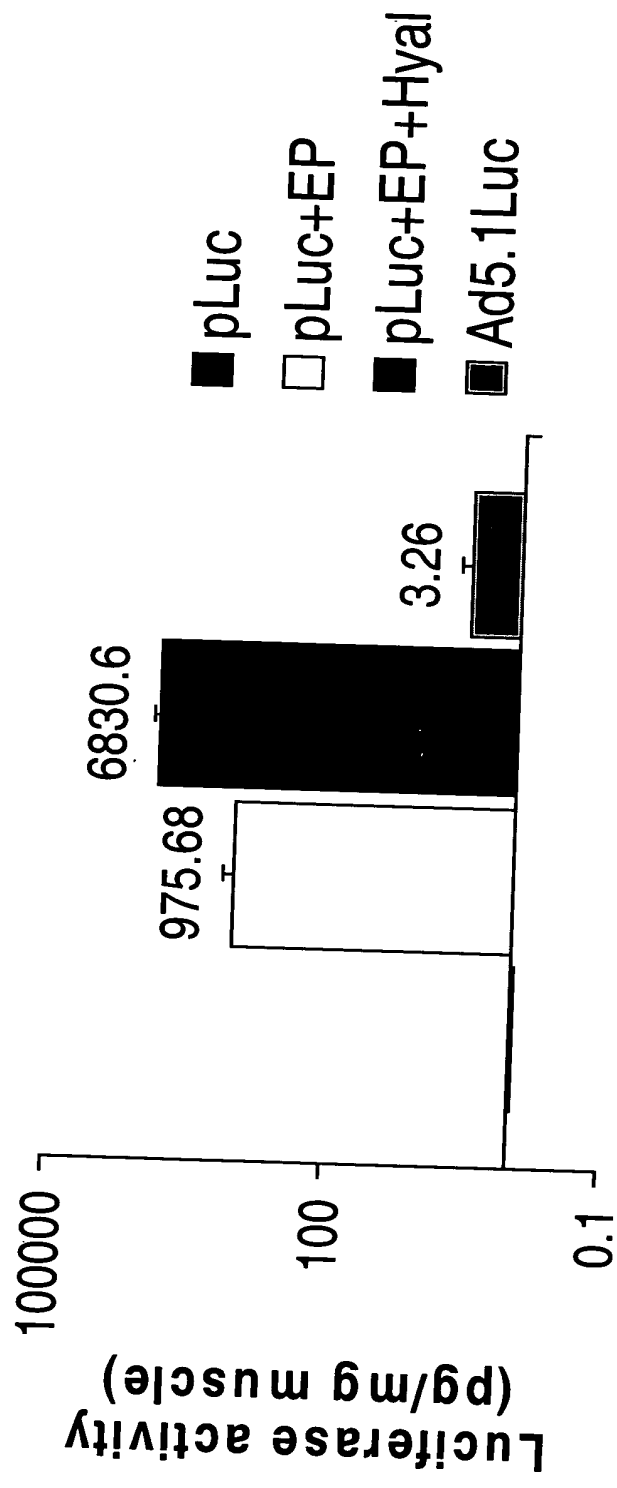


11-12 month old ecNOS-KO mice at day 10



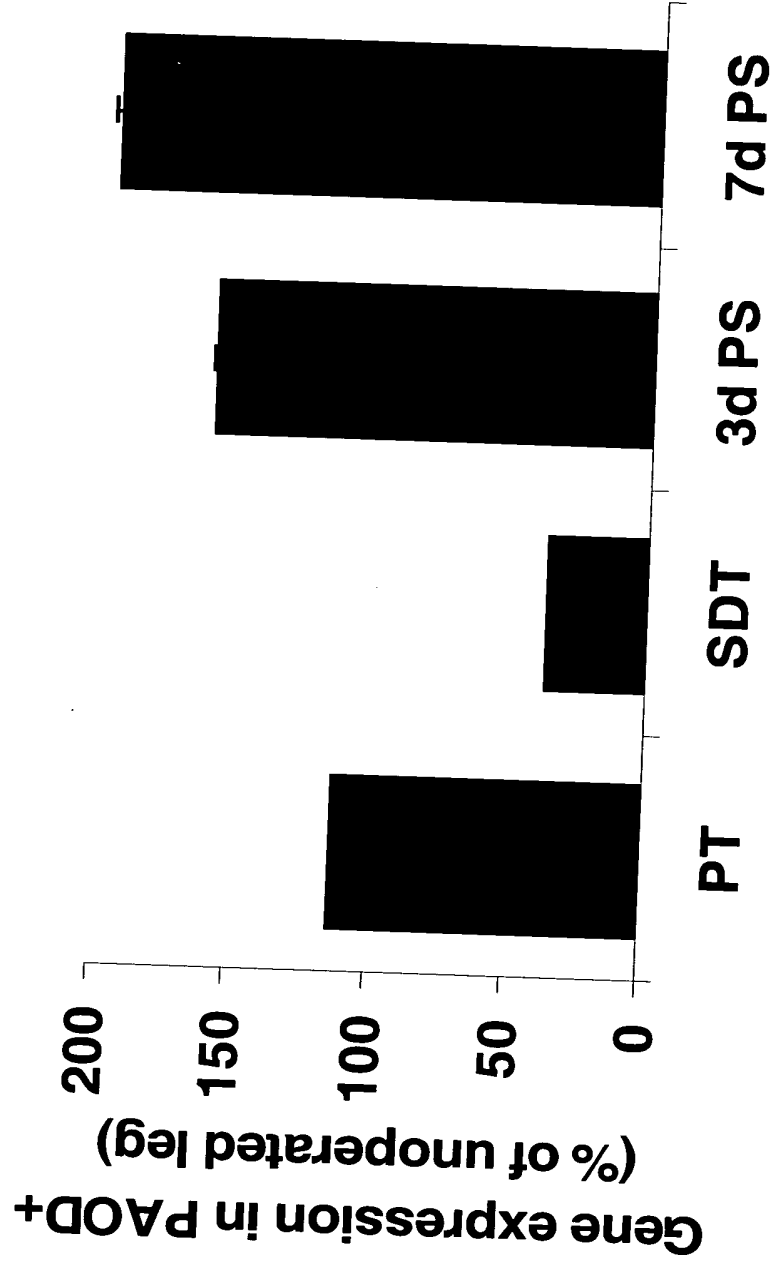
10/28

FIGURE 10



11/28

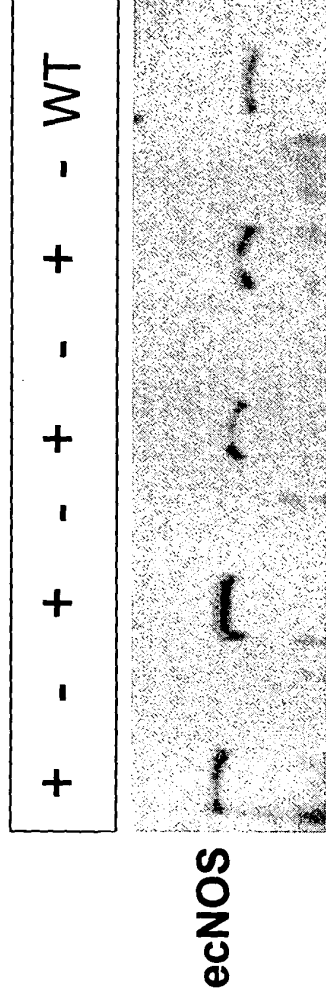
FIGURE 11



12/28

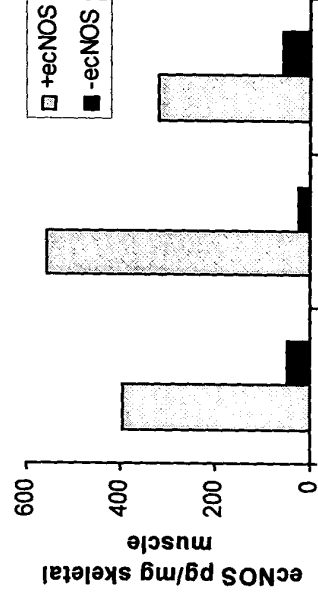
FIGURE 12: ecNOS expression in ecNOS-KO mice following gene therapy

Western blot for ecNOS



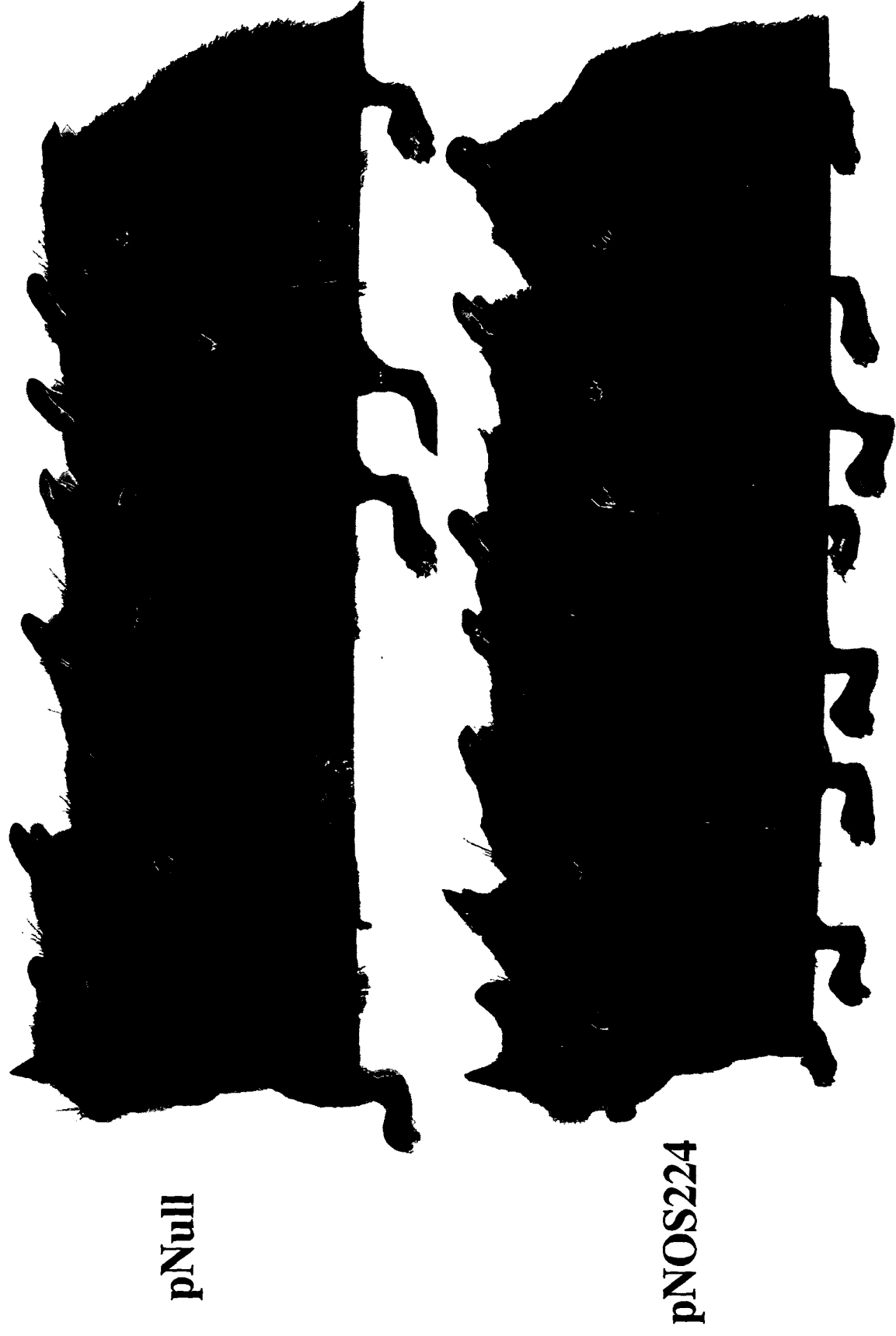
ecNOS ELISA

(ecNOS-KO mice, pNOS224+EP)



13/28

FIGURE 13: Limb salvage after pNOS224 delivery in 6 month old ecNOS mice.



14/28

FIGURE 14

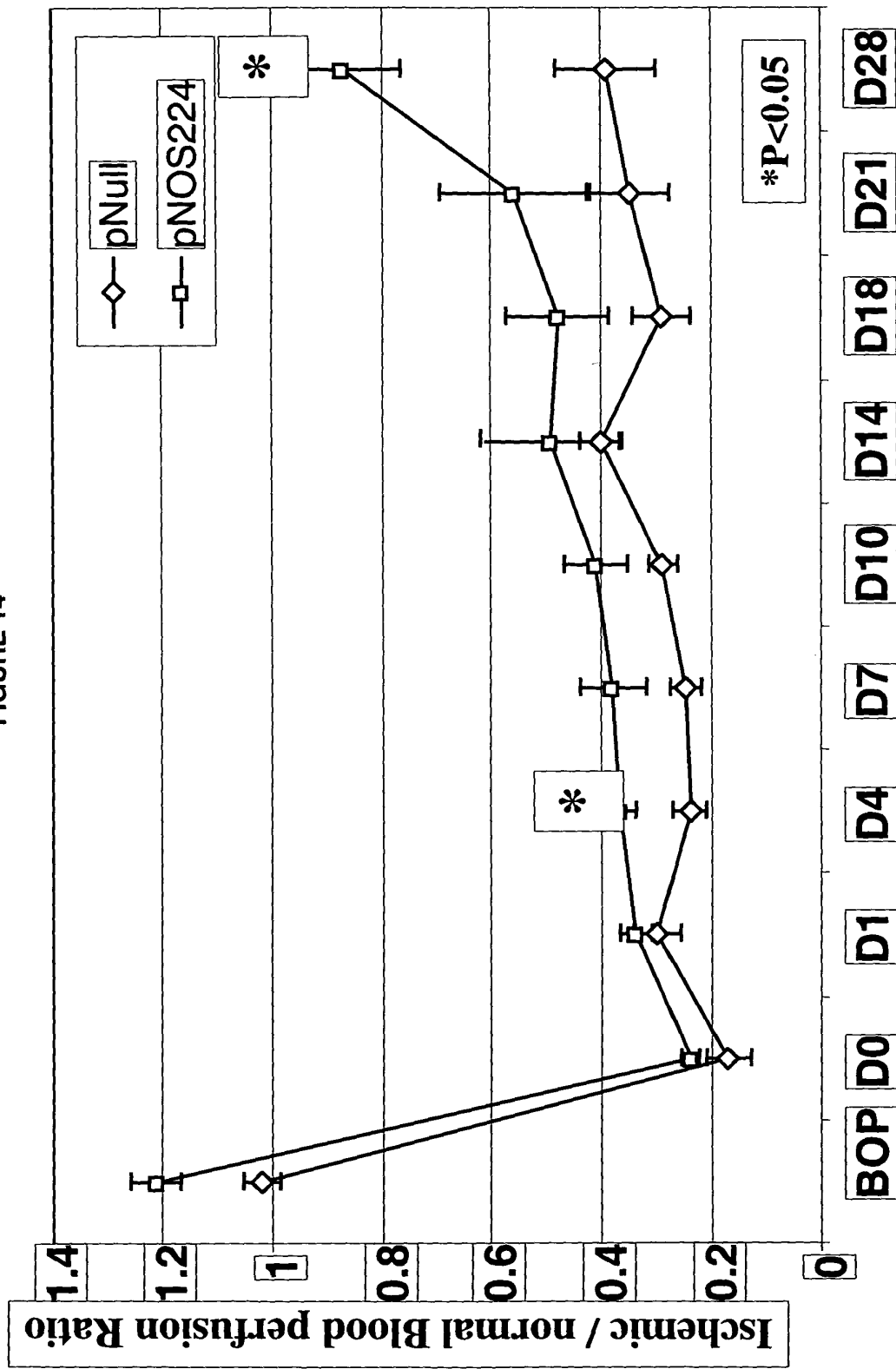


FIGURE 15: Histomorphometric analysis of pNOS224 treated hindlimbs vs. empty vector treatment

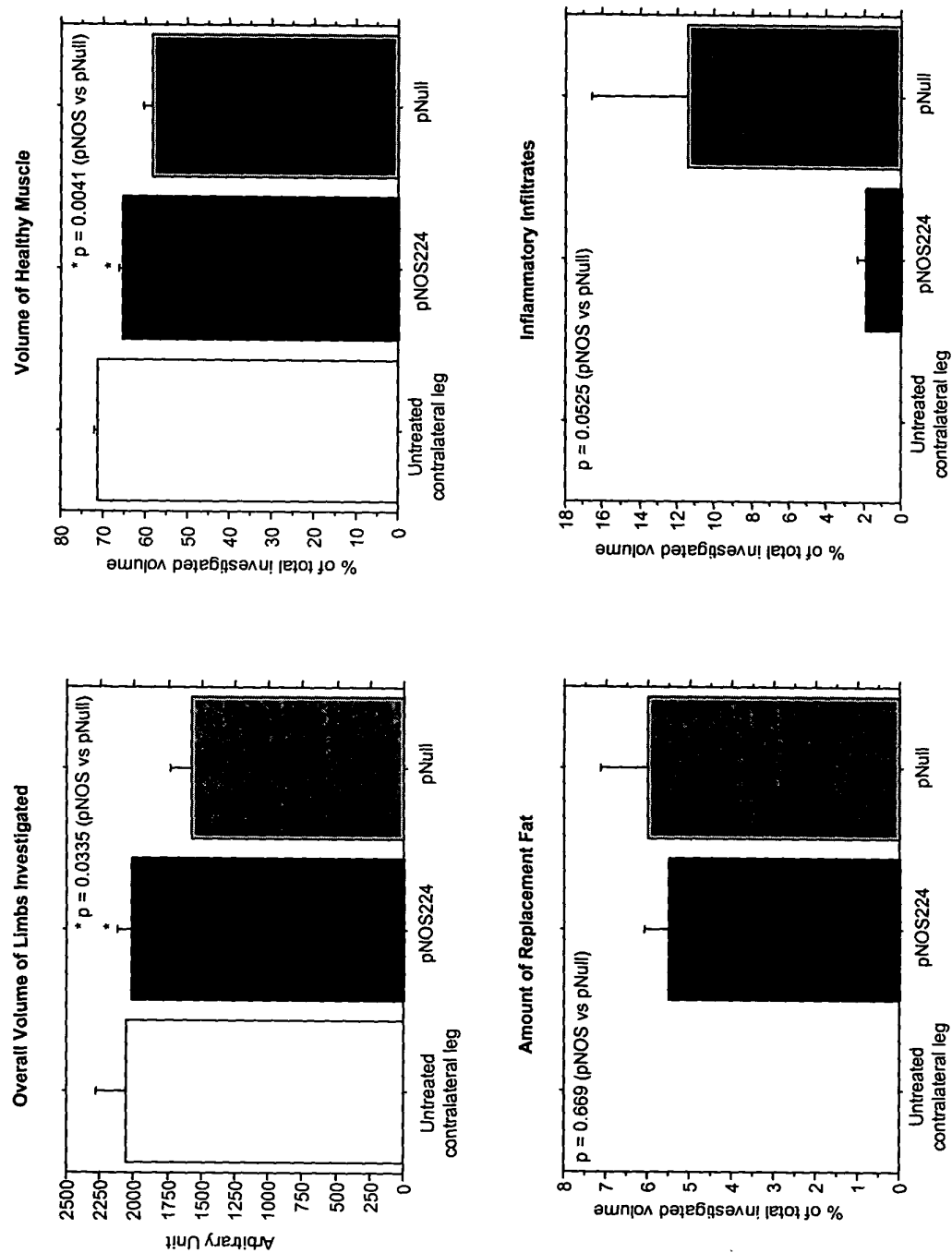
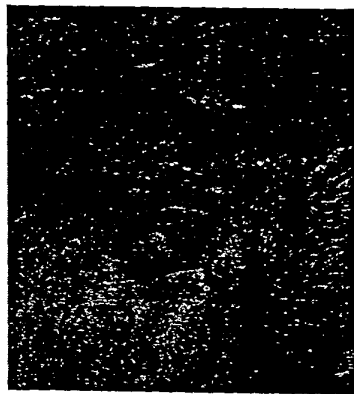
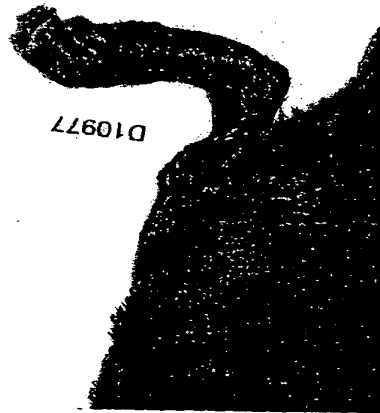
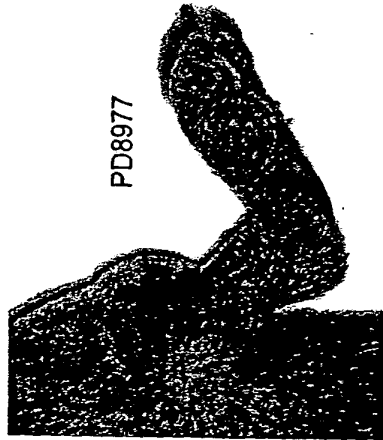
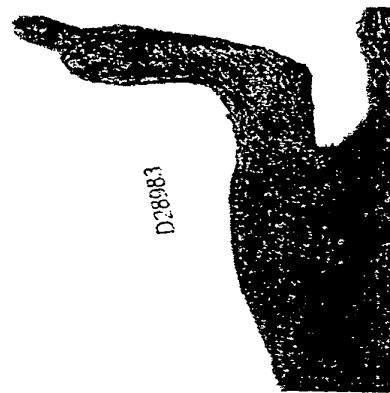
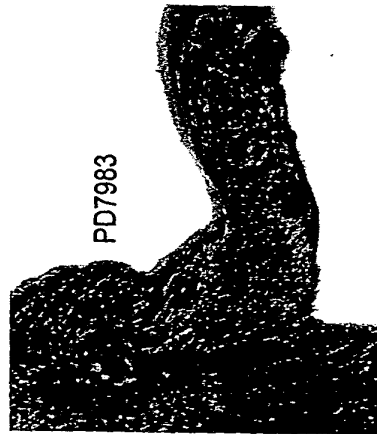


FIGURE 16: Healing of skin ulcers in pNOS224 treated mice

pNull



pNOS224



17/28

FIGURE 17: Limb salvage in 11-12 month old mice

pNull

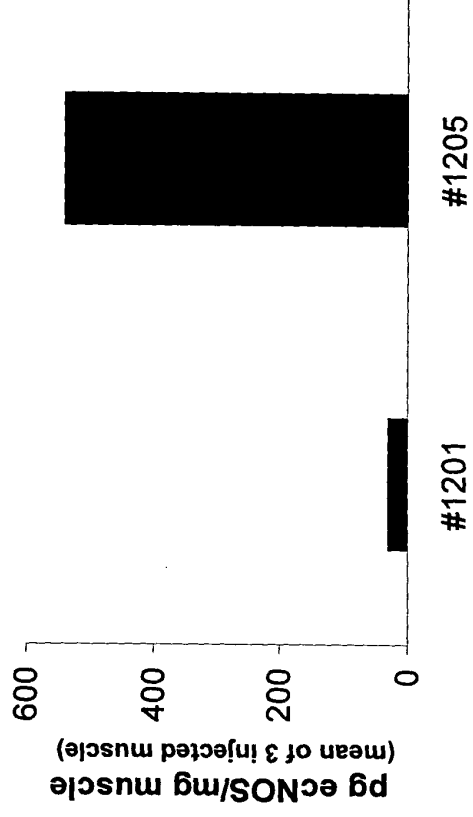


pNOS224

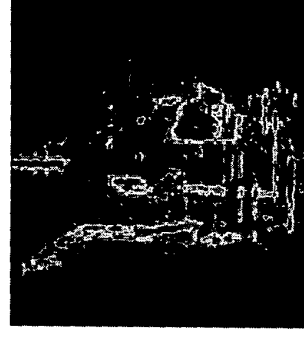
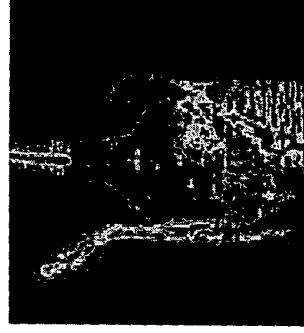


18/28

FIGURE 18: ecNOS protein expression and therapeutic effect of gene delivery



ecNOS PROTEIN
EXPRESSION



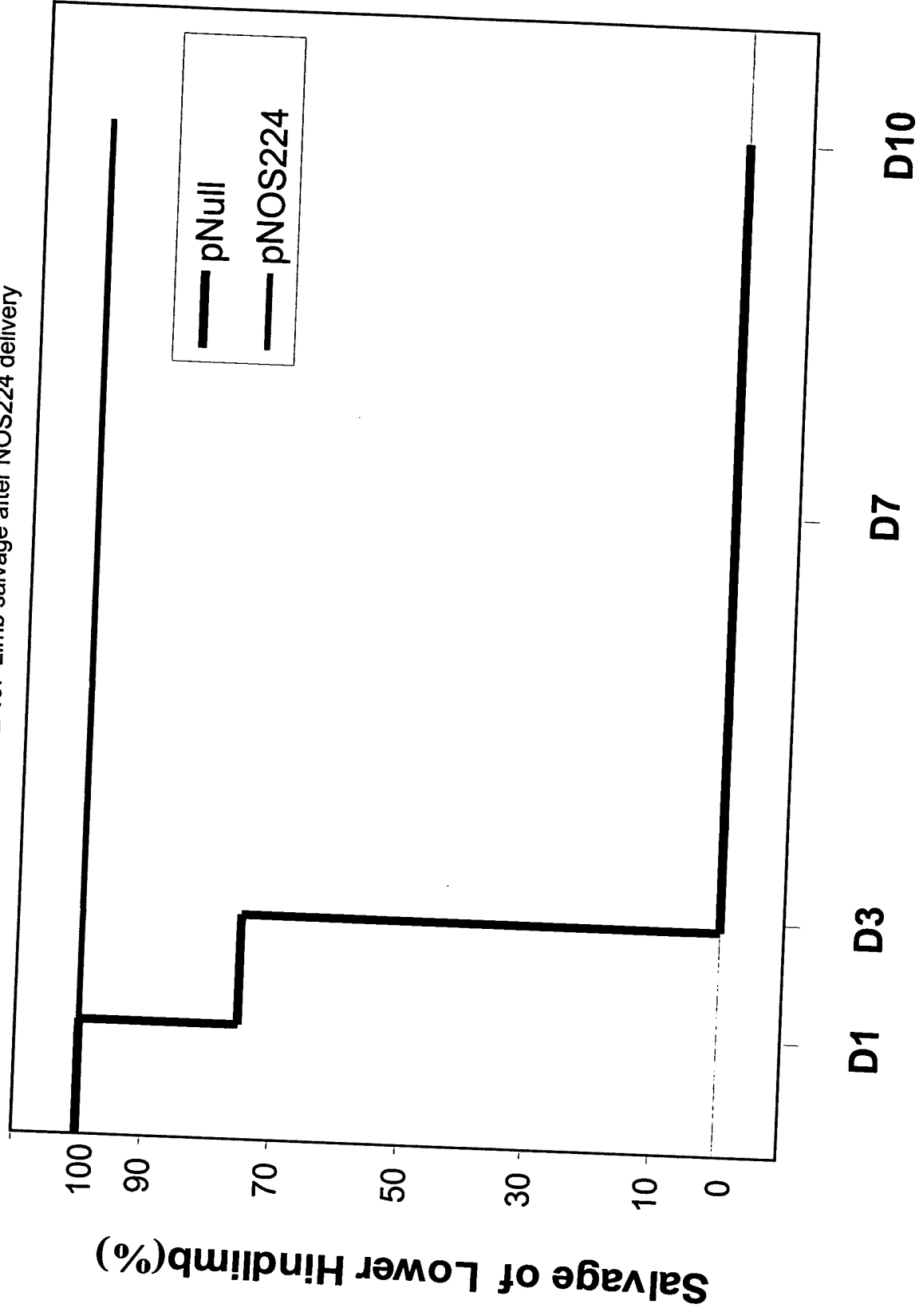
BLOOD FLOW
(LDPI)



LIMB NECROSIS

19/28

FIGURE 19: Limb salvage after NOS224 delivery



20/28

FIGURE 20: Effect of NOS224 treatment on LDPI measured flow

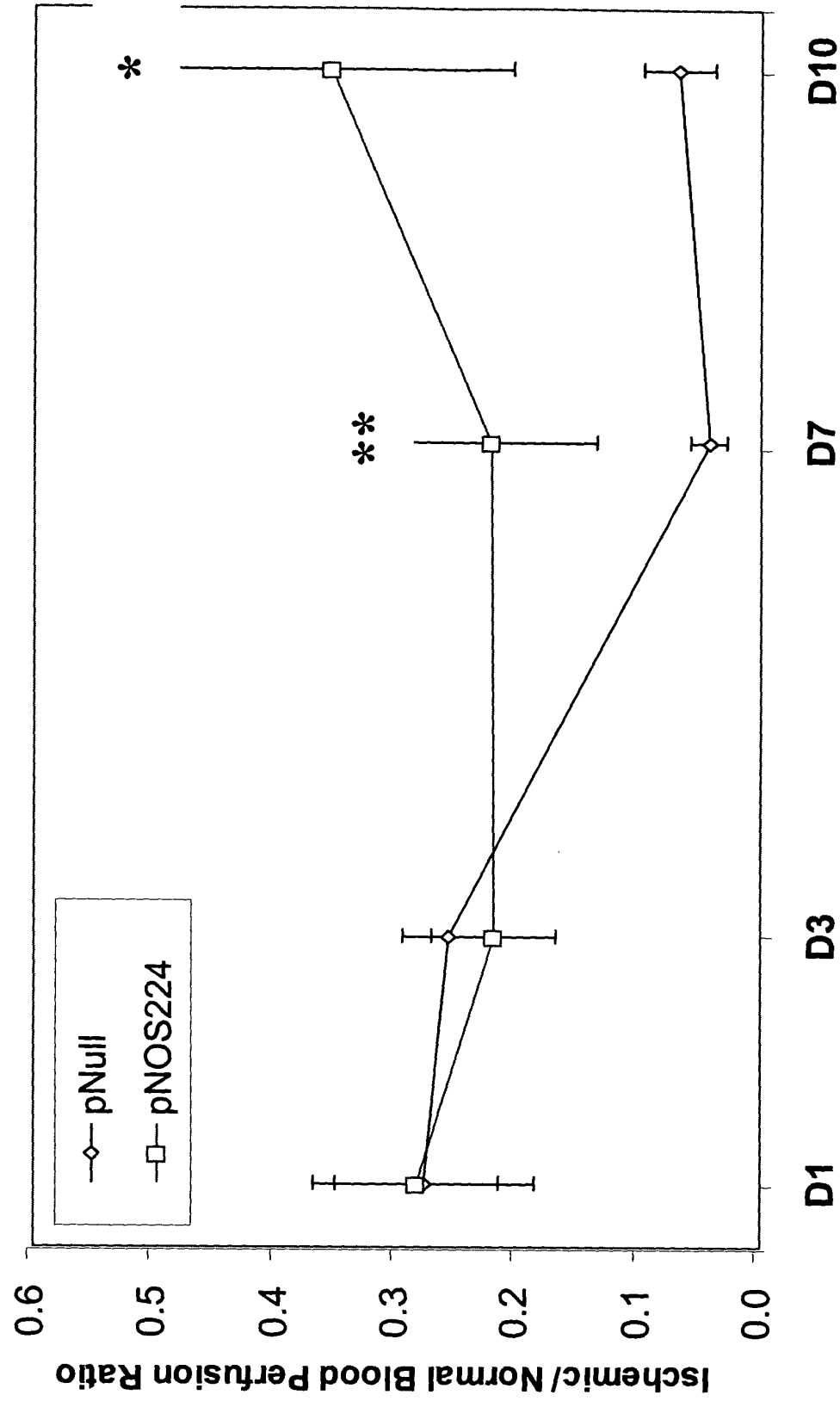


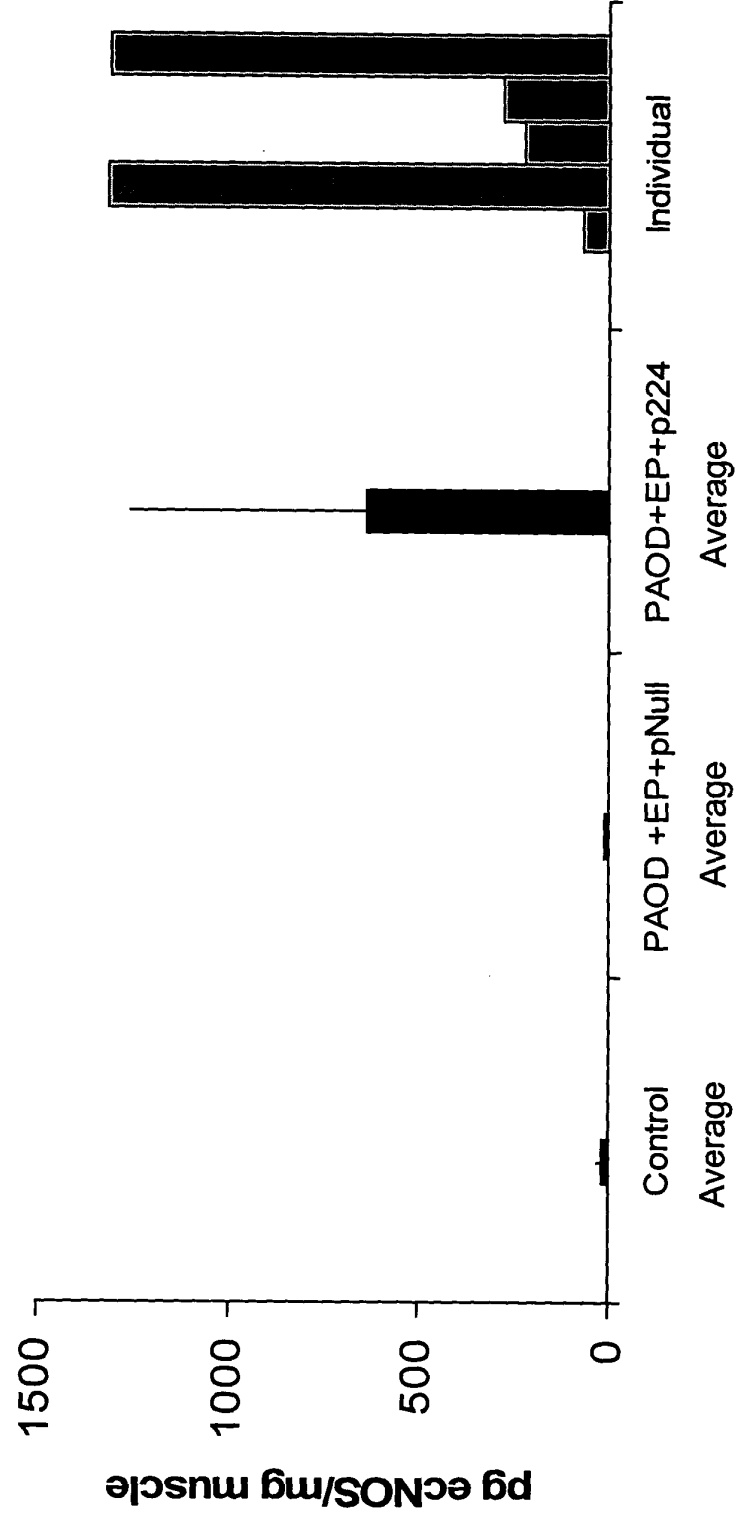
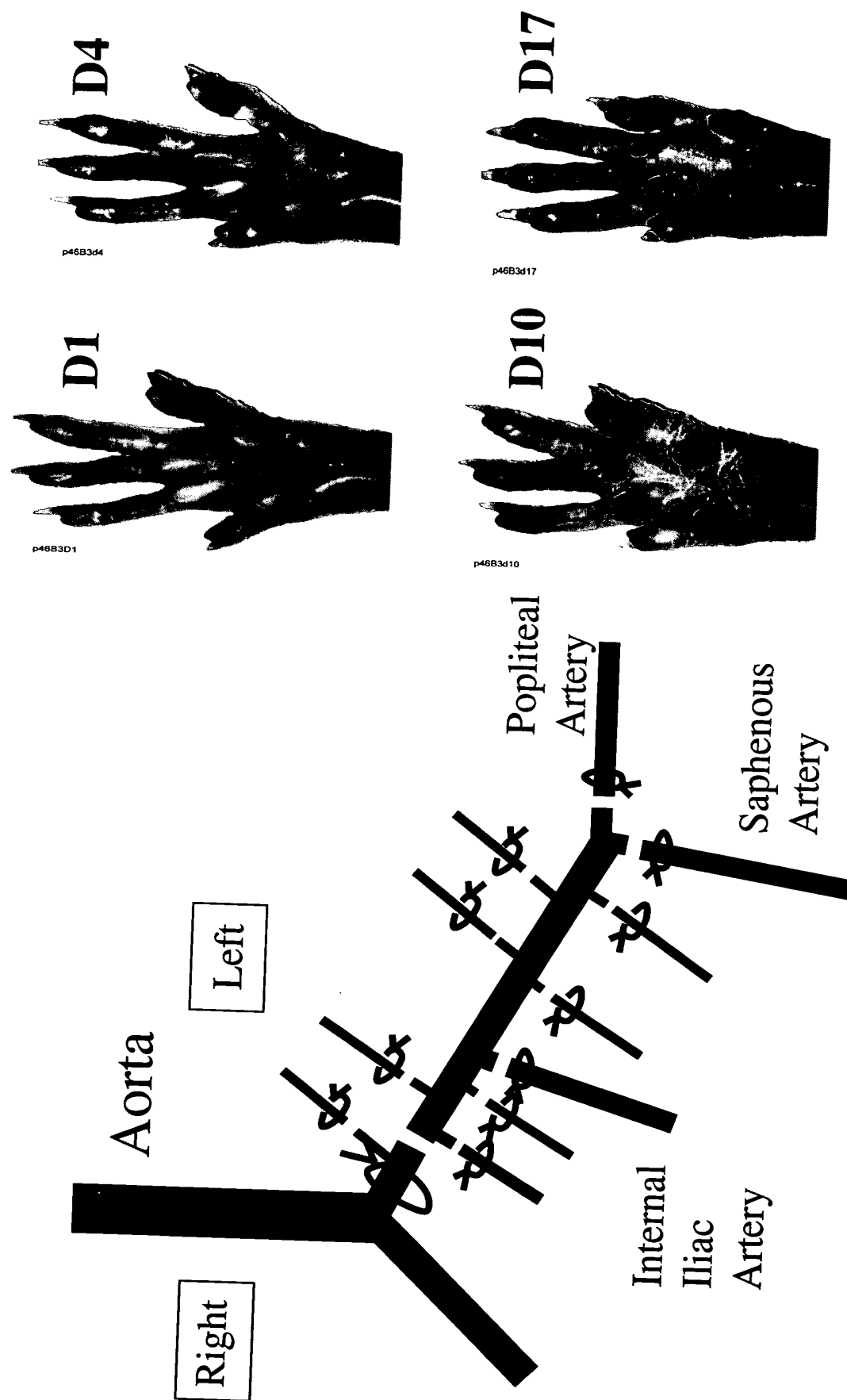
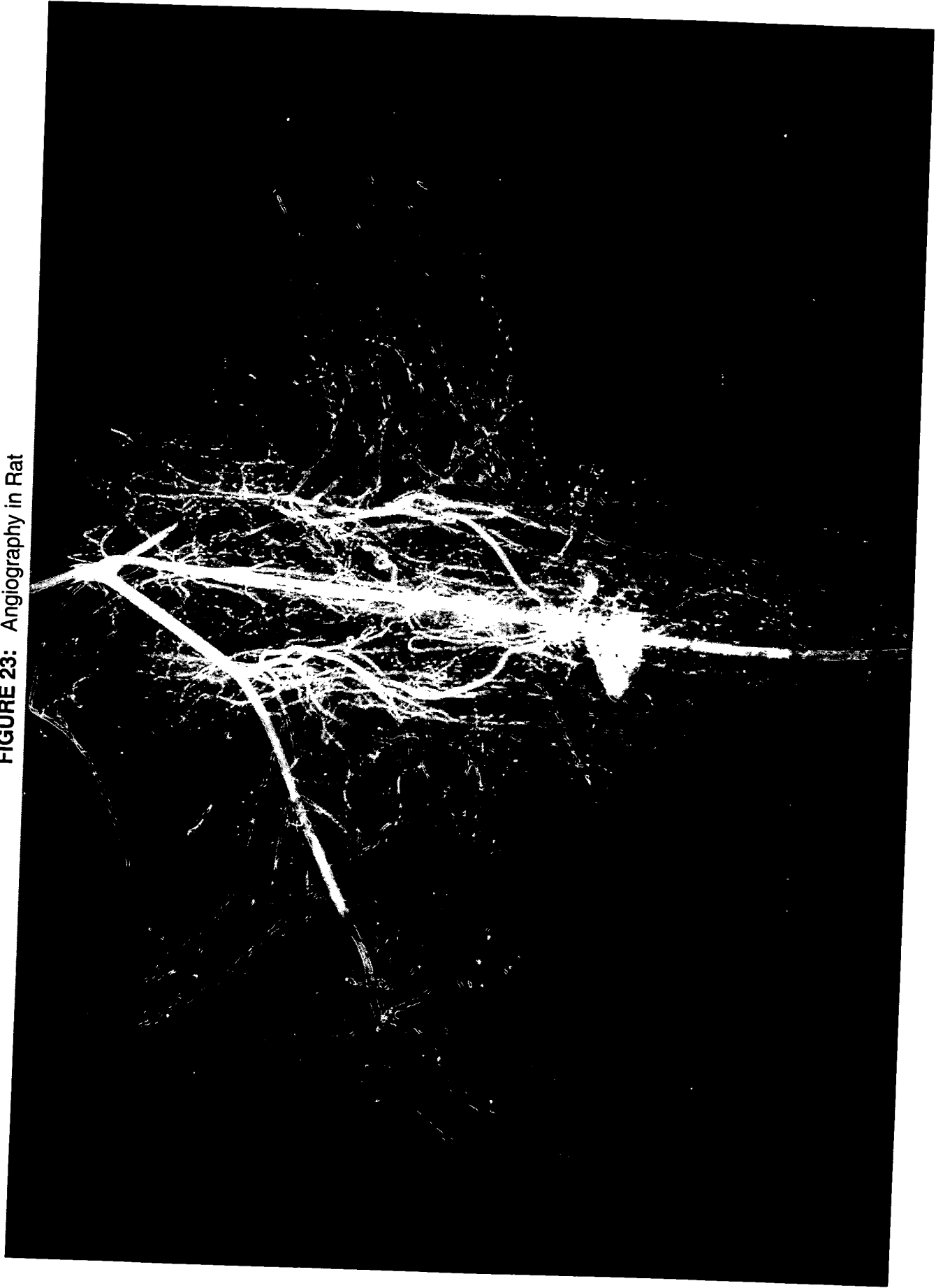
FIGURE 21: ecNOS protein expression in the adductor muscle pf ecNOS-KO mice

FIGURE 22: CLI Rat Model



23/28

FIGURE 23: Angiography in Rat



24/28

FIGURE 24: Angiographic Score



To quantitate arteriogenesis, three straight lines started from internal 1/4, middle, and external 1/4 of the femur were drawn at the medial thigh area of both normal and ischemic limb, and total number of arteries crossing these lines were calculated by **two separate investigators blinded to the treatment.**

To minimize variations, angiographic score was expressed as the ratio of total artery number of left to right hindlimb.

FIGURE 7: Blood flow recovery after Ad5NOS delivery

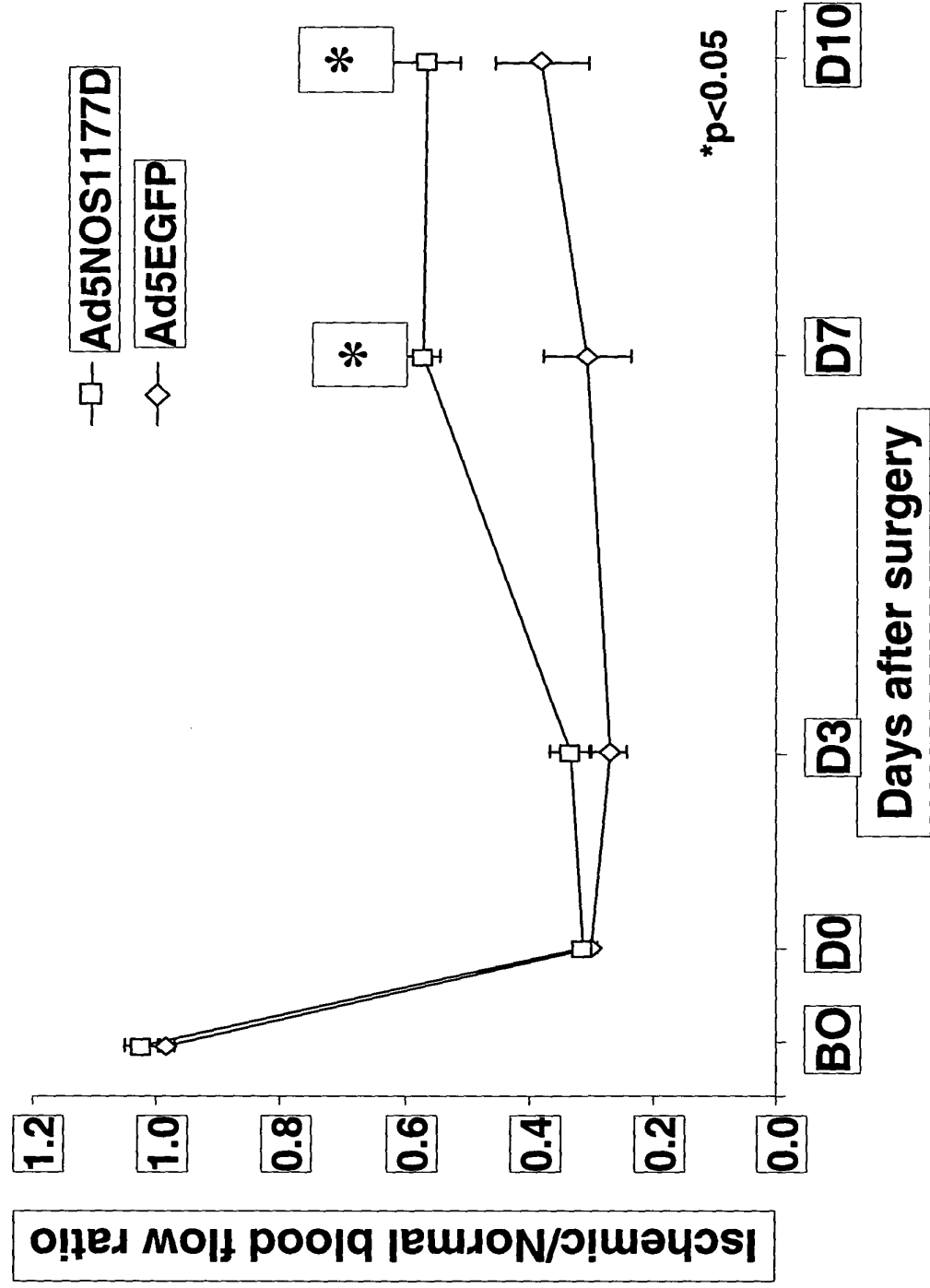


FIGURE 26: NECROTIC SCORE






STAGE		GROSS PATHOLOGY	SCORE
Stage I		Normal situation or nail necrosis	0
Stage II		Toe(s) necrosis or discoloration	1
Stage III		Toe(s) loss or paw necrosis	2
Stage IV		Loss of all toes or partial paw	3
Stage V		Loss of more than half paw	4

FIGURE 27

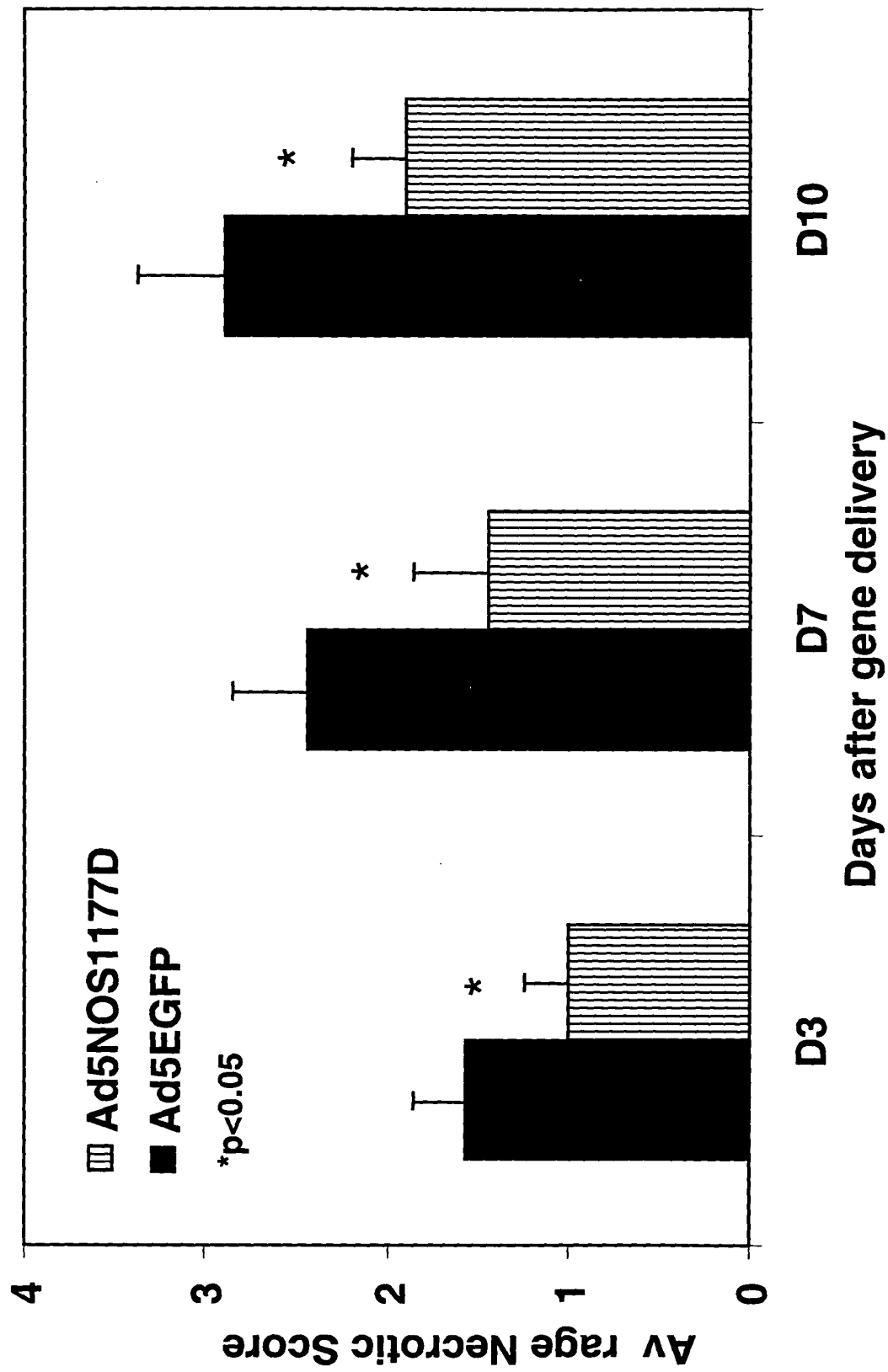


FIGURE 28

